Determination of Acidity, Nitrogen, and Ash in Honey*

A recently described method for determining free lactone and total acidity of honey (*This Journal,* **41**, 194 (1958)) was studied collaboratively in comparison with the official method (*Official Methods of Analysis,* 9th Ed., 1960, sec. **29.131**).

Three samples of honey were selected to cover the normal range of honey acidity. These samples were analyzed in triplicate by the official method and by the proposed method. Five collaborators participated and all reported; in addition the Associate Referee and a colleague analyzed the samples.

METHODS

Total Acidity.—The collaborators were instructed to use method **29.131**, the official method for free acid, with triplicate determinations, and the proposed procedure also in triplicate, as follows:

Dissolve 10 g sample in 75 ml CO_2-free H_2O in 250 ml beaker. Stir with magnetic stirrer, and immerse electrodes of pH meter in soln and record pH. Titr. with 0.05*N* NaOH at rate of 5.0 ml/min. Stop addn of NaOH at pH 8.50. Immediately pipet in 10 ml 0.05*N* NaOH and immediately back-titr. with 0.05*N* HCl from 10 ml buret to pH 8.30. Calc. as milliequiv./kg: Free acidity = (ml 0.05*N* NaOH from buret − ml blank) × 50/g sample; lactone = (10.00 − ml 0.05*N* HCl from buret) × 50/g sample; total acidity = free acidity + lactone.

Ash in Honey.—The procedure for the determination of ash in honey was applied to 500 samples of honey in this laboratory. The method is as follows:

Weigh 5–10 g honey into ignited and weighed Pt dish. Place under 375 watt infrared lamp with variable voltage input and slowly increase applied voltage until sample is black and dry and there is no danger of loss by foaming. Heat in muffle at 600° to constant wt (overnight). Cool and weigh.

Nitrogen in Honey.—No method is specified in *Official Methods of Analysis* for determining nitrogen in honey. The following procedure is essentially that of **38.009–38.011**, with slight modification:

Det. N as in **38.009–38.100**, using 300 mg sample, 3.0 ± 0.1 ml H_2SO_4, and 1 hr digestion after acid comes to true boil. Titr. with 0.01*N* HCl and calc. % N.

Invert Sugar in Honey.—The resorcinol test (procedure) for commercial invert sugar, **29.122**, was revised to read:

Dissolve 2 g honey in 10 ml H_2O and ext. rapidly with washed ether 30 min. in continuous extractor, Fig. 63-B, p. 475. Conc. ether to ca 5 ml and transfer to test tube. Add 2 ml freshly prepd resorcinol soln, shake, and note color. Cherry red color appearing within 1 min. indicates presence of commercial invert sugar. Yellow to salmon shades have no significance.

The aniline chloride test, **29.123–29.124**, was deleted.

Results and Discussion

Results on the determination of acidity in honey are shown in Table 1. Because Collaborator G consistently reported negligible lactone values, his results were not included in calculation of the averages or the standard deviations.

In general, these results were somewhat higher (about 1–2 milliequivalents per kilogram) than those obtained by the official method. This was expected from the rapidity of the proposed free acid titration. The lactone values by the proposed method represent "reserve" acidity; the official method does not measure "reserve" acidity, although lactone is responsible for the obscure end point. The final end point by the proposed procedure was stable in contrast to that of the official method. Thus, total acidity by the proposed method should be more reproducible between laboratories. Comparison of the appropriate standard deviation does show this tendency in 2 of the 3 samples, but it is not statistically significant. The effect on reproducibility of the (purposely)

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Table 1. Determination of honey acidity[a]

Coll.	Official Method	Proposed Method		
		Free	Lactone	Total
Sample 1				
A	12.8	13.3	6.8	20.1
B	11.0	11.9	6.1	18.0
C	10.6	10.7	6.8	17.6
D	11.5	12.6	4.9	17.6
E	15.2	18.1	3.0	21.1
F	11.5	14.1	4.6	18.7
G[b]	16.6	16.3	0.3	16.6
Av.	12.1	13.4	5.4	18.8
Std Dev.	1.65	2.56	1.49	1.45
Sample 2				
A	16.2	16.5	7.3	23.3
B	15.0	15.8	6.9	22.7
C	13.6	13.6	8.0	21.6
D	15.4	16.7	6.4	23.1
E	19.7	21.8	4.1	25.8
F	15.3	18.7	4.6	23.3
G[b]	17.1	18.2	0.3	18.5
Av.	15.9	17.1	6.2	23.3
Std Dev.	2.06	2.87	1.54	1.38
Sample 3				
A	26.6	26.4	11.7	38.0
B	22.8	25.0	13.0	38.0
C	21.6	22.7	10.9	33.6
D	24.2	26.4	10.8	37.3
E	27.2	30.6	9.9	40.5
F	26.1	28.7	10.3	38.9
G[b]	35.5	29.3	0.4	29.7
Av.	24.7	26.6	11.1	37.7
Std Dev.	1.96	2.78	1.11	2.30

[a] Expressed in milliequivalents per kilogram. Each value is an average of triplicates.
[b] These values were excluded from calculations.

rapid free acid titration in the proposed method can be seen from the consistently higher standard deviation values.

The precisions of the official method and of the proposed total acidity determination are shown in Table 2. The pooled value shows that standard deviation by all analysts is of the same order for both methods. The standard deviation values indicate the wide range of precision obtained by the analysts, only two of whom were familiar with either procedure. The results of Collaborator F show the potentiality of the procedures.

Although determination of the pH of honey is not presently an AOAC method, it gives a useful value and should be included in the procedure. Since the proposed procedure specified a pH meter for end point detection, collaborators were requested to determine the pH of honey solution before titration. As pH cannot be averaged, representative values for each collaborator are shown in Table 3. The range of values for each sample is somewhat higher than was expected.

Table 2. Precision of acid determination

Coll.	Standard Deviation of Individual Values	
	Official Method	Proposed Method[a]
A	0.24	0.71
B	0.46	0.13
C	0.29	0.80
D	0.26	1.11
E	1.61	1.29
F	0.11	0.21
G	0.87	0.68
Pooled Value	0.88	0.97

[a] Total acidity.

It has been noted in this laboratory that the pH meter used for the proposed method must be correct at both pH 4 and 8; calibration at pH 8 is essential for the titration. The pH 4 calibration is required for accurate pH determination on honey. Use of only the pH 4 calibration for the entire determination can lead to inaccurate titration values; reliance on the pH 8 calibration alone can yield erroneous pH values. The collaborators were not given specific calibration instructions, and no information on their practices is available.

Comments of Collaborators

Collaborator E was unable to get good checks by either method. For the proposed method, he used six or seven replicates of each sample; only the last three were reported. He used a pH meter for the official method.

Collaborator F noted some variation between the triplicates of Samples 1 and 2 for the milliequivalent per kilogram value of the free acid and lactone present. He found this variation to be much less in Sample 3. However, his results for the total acidity (free acid plus lactone) between the triplicates of each sample agree fairly well.

Table 3. Determination of pH of honey

Coll.	Sample		
	1	2	3
A	3.80	4.04	3.78
B	3.90	4.10	3.80
C	3.82	4.06	3.82
D	4.11	4.30	4.07
E	3.85	4.08	3.92
F	4.08	4.18	3.96
G	3.68	4.01	3.70

Collaborator F believed that the variation in the values for free acid and lactone between the triplicates of Sample 1 and 2 was due to the rate of addition of the NaOH to the first end point, pH 8.50. The NaOH was added as suggested in the method until the end point was approached, and then it was added dropwise until the final end point was reached. Thus, according to Collaborator F, a varying amount of lactone could have been titrated in Samples 1 and 2 before reaching the end point at pH 8.50. As evidence, he points out that the lactone was higher and the total acids agreed more closely for those samples in which the free acid was lower. Collaborator F found that when the triplicates of Sample 3 were titrated rapidly with NaOH to the first end point (pH 8.50), the values for free acid and lactone agreed more closely. (All of Collaborator F's titrations were carried out with a Fisher Titrimeter.)

Collaborator F mentioned the difficulty of determining when all the free acid was titrated without any lactone being titrated. He felt that the total acid value as determined by the proposed method would be a more satisfactory value to be used and should be more reproducible. For this reason, and from comparison of results by the two methods, he preferred the proposed method.

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Recommendations[1]

It is recommended—

(1) That the method for free acid in honey, **29.131**, be deleted.

(2) That the proposed method for free, lactone, and total acidity, described in this report, be adopted as first action.

(3) That method **29.097** for ash in honey be modified as described in this report.

(4) That the method described in this report for determination of nitrogen in honey (**38.009–38.011**) be adopted as first action.

(5) That the actions of the Association in 1932 regarding methods for the detection of invert sugar in honey be appropriately noted.

(6) That collaborative work on the first action selective adsorption method for honey sugars, **29.107–29.116**, be continued.

(7) That the first action Schade method for determination of diastatic activity of honey, **29.125–29.130**, be further studied.

(8) That the proposed method for determining free, lactone, and total acidity in honey be further studied.

[1] These recommendations were approved by the General Referee and by Subcommittee D, and were adopted by the Association. See This *Journal*, **45**, 128 (1962).